REMARKS

After entry of the present amendments Clams 1-9, 11-15, 18, 19, 22, 24, and 31-34, 36-49, 51-63, 65-82 will be pending. Claim 1 is amended herein and new Claims 80-82 are added. Claim 1 is amended to recite forming a cheese that can be frozen and thawed while still maintaining a smooth texture. New Claims 80-82 are added reciting various fat and water contents of the cheese. Support for the amendments can be found throughout the specification as originally filed, for example at paragraphs [0010], [0073], [0116], [0124], and [0131] of the specification as published. No new matter is added.

Applicants thank the Examiner and her Supervisor for taking the time to interview and discuss the application after the final Office Action.

Rejections under 35 U.S.C. § 112

The Examiner rejected Claims 1-4 under 35 U.S.C. § 112, first paragraph for failing to comply with the written description requirement. The Examiner found that the protein concentrate selected from coagulated rennetted milk protein concentrate, a coagulated rennetted milk, or a reconstituted coagulated rennetted milk protein concentrate was not disclosed in the specification.

Applicants note that at paragraph [0051] of the application as published the specification discloses that one option for the protein concentrate is a milk protein concentrate that has been formed from rennetted milk. In addition, at paragraphs [0068]-[0076] of the application as published a process is described for preparing a protein concentrate using rennetted skim milk or reconstituted milk powder (MPC). Although it may not be stated explicitly one of skill in the art would appreciate that the coagulated rennetted milk is clearly coagulated protein formed by rennet, i.e. a coagulated rennetted material. The specification clearly provides support for the protein concentrate being selected from coagulated rennetted milk protein concentrate, a coagulated rennetted milk, and a reconstituted coagulated rennetted milk protein concentrate. Accordingly, Applicants submit that coagulated rennetted milk protein concentrate, a coagulated rennetted milk, and a reconstituted coagulated rennetted milk protein concentrate as recited in Claims 1-4 are clearly supported in the specification.

The Examiner also found that the change from "coagulated cheese mass" to "homogenous cheese mass" suggests that Applicants are claiming that coagulation happens by the action of the rennet only, but that this was not described in the specification as filed. However, as would be understood by a person of skill in the art, coagulation can occur by processes other than enzyme coagulation. The term "coagulated cheese mass" as recited in step (c) of claims 1-4 does not refer to ***rennet*** coagulation but to ***heat*** coagulation; the claims themselves recite heating and mixing. Heat coagulation would be understood by the skilled artisan to include forming a homogenous cheese mass. The specification clearly discloses coagulation of protein and other ingredients by mixing and heating. Accordingly, Applicants submit that the "homogenous cheese mass" as recited in Claims 1-4 is clearly supported in the specification.

For the reasons discussed above, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112.

The Combination of Johnston/Lashkari alone or with Bernard Does Not Make Claims 1-5, 8, 9, 11-13, 15, 18, 19, 22, 31, 34, 36-38, 40-43, 45, 49, 51-53, 55-58, 62, 63, 65-67, 69-72, 74, and 76-79 Obvious

Claims 1, 4, 5, 9, 11-13, 15, 18, 19, 22, 63, 65-67, 69-72, 76, and 79 stand rejected under 35 U.S.C. § 103(a) as unpatentable in view of WO 03/069982 to Johnston (hereinafter "Johnston") and GB 1,057 170 to Lashkari (hereinafter "Lashkari").

Claims 2, 3, 8, 31, 34, 36-38, 40-43, 45, 49, 51-53, 55-58, 62, 74, 77, and 78 stand rejected under 35 U.S.C. § 103(a) as unpatentable in view of Johnston, Lashkari, and U.S. Patent No. 4,948,613 to Bernard et al. (hereinafter "Bernard").

The Examiner found Johnston to disclose a process for making cheese using a protein concentrate that could be a milk protein concentrate, milk, or reconstituted milk that is exposed to rennet and is coagulated to produce curd. The Examiner further found that Johnston disclosed mixing in natural flavors to disperse the flavor in the curd (pg. 11, lines 1-17). The Examiner found that Johnston does not disclose providing a flavor concentrate using at least one strain of organism. The Examiner found Lashkari to disclose a cheese flavor composition containing an edible mold which is *P. roqueforti* that can be added to a food composition to provide a cheesy

flavor. The Examiner found that it would have been obvious to use the cheese flavoring composition of Lashkari in the process of Johnston.

The Examiner found Bernard to disclose a cheese product that is cooled with the surface of the cheese inoculated with micro-organisms that grow and promote ripening of the cheese (col. 4, lines 48-55). The Examiner further found that it would have been obvious to combine Bernard with the processes of Johnston and Lashkari.

The combination of references fails to disclose or make obvious the features of Claims 2 and 4 and their dependents

Claims 2 and 4 recite inactivating the flavour producing organisms. The combinations proposed by the Examiner fail to disclose these features as claimed. Claims 2 and 4 recite inactivating the flavour producing organisms. Claim 2 recites in part "heating and mixing to form a homogeneous cheese mass without holding for fermentation, and ***inactivating the flavour producing organisms***". Claim 4 recites in part providing a (b) flavour concentrate using at least one strain of organism, and (c) heating and mixing to form a homogeneous cheese mass without holding for fermentation, and ***inactivating the flavour producing organisms***." The cited references fail to disclose inactivation.

Inactivation means essentially no longer producing flavor and so enzymes are necessarily inactivated. On the other hand, to a person skilled in the art, sterilize means to render the organisms therein non-viable and does not include inactivating the flavor producing activity (i.e. enzymes). *See* Heat Resistant Proteolytic Enzymes from Bacterial Sources by Speck and Adams, a copy of which is attached to this response and submitted concurrently in an IDS, ("There now is growing evidence that enzymes capable of spoiling milk can survive such UHT treatments." and "The time required to reduce protease activity by 90% was 90 s compared to .25 s and .02 s for a 90 % reduction in PA3679 spores and *B. stearothermophilus* spores, respectively." at page 786). In contrast to sterilization, a person of skill in the art would understand inactivation to include inactivating enzymes present in the treated material. It is also well known that heat treatments required to destroy enzymes are considerably in excess of the heat treatments necessary to kill the associated host microorganisms. *Id.* at 786.

Johnston fails to disclose inactivating organisms as recited in the claims or conditions that would inactivate organisms. Johnston discloses that flavor ingredients may comprise various fermentation and or enzyme derived products or mixtures thereof (page 11, lines 8-9). Fermentation and/or enzyme derived products are mixtures that are different from fermentates because fermentates can have active bacteria and enzymes present. The Examples in Johnston disclose products without active bacterial cultures or enzymes. For example, Johnston discloses that "[t]he flavours comprised a mixture of pre-prepared concentrated fermentation and enzyme-derived flavour ingredients [1.5% Alaco EMC (DairyConcepts, USA), 350 ppm Butyric acid and 16 mM acetate in final product (Bronson & Jacobs Ltd, NZ)." (page 12, lines 24-26). Johnston further discloses that "[t]he flavours comprised pre-prepared concentrated fermentation and enzyme-derived flavour ingredients [50 ppm Butyric acid, 8 mM acetate and 2.5 ppm diacetyl in the final product (Bronson and Jacobs Ltd, NZ) and 1 ppm Lactone." (page 13, lines 27-29). These flavoring products are derived from fermentation or enzymes products and they are not enzymes or bacteria themselves. Thus, these flavoring components do not have active bacteria or enzymes.

Johnston further discloses heating and mechanical working of the cheese at a temperatures of between 50°C and 90°C when stretched in hot water or 50°C to 75°C when stretched in a dry environment after adding flavor compounds (page 9, lines 30-page 10, line 6). A person of skill in the art would appreciate that such temperatures are roughly equivalent to pasteurization conditions and would not be sufficient for inactivation. *See* Speck and Adams. Most organisms cannot survive these conditions; however, enzymes can remain viable. Therefore the conditions in Johnston are insufficient for inactivation of organisms as claimed and thus Johnston fails to disclose inactivation explicitly or inherently. Johnston fails to disclose inactivating flavor producing organisms as recited in Claims 2 and 4. Further, this more than an obvious variation because the flavor concentrates disclosed in Johnston do not have enzymes present and thus there would be no need to perform an inactivation step. Johnston fails to provide any reason for inactivation.

Lashkari also fails to disclose inactivation. Lashkari teaches the production of a cheesy flavored fermentate using lipolytic micro-organisms (page 1, line 18). Lashkari further teaches that the flavorful fermentate may be sterilized (page 2, line 44). Lashkari fails to disclose

inactivation of the flavor producing organisms. In addition there is no teaching in Lashkari to produce a flavor stable cheese where the enzymes in the flavored fermentate would need to be inactivated.

The Examiner found Bernard to disclose a cheese product that is cooled with the surface of the cheese inoculated with microorganisms (Office Action at page 6). Bernard fails to disclose inactivation or make up for the deficiencies of Johnston and Lashkari that are noted above.

The skilled artisan would appreciate that the heating steps of Johnston and sterilization of Lashkari would not necessarily stop the enzyme activity. Thus, the combination of Lashkari, Johnston, and Bernard does not disclose explicitly or inherently or make obvious the features of Claims 2 and 4 and their dependents. Accordingly, Applicants respectfully request withdrawal of the rejections of Claims 2 and 4 for at least this reason.

There would also be no reason to combine Johnston and Lashkari or Johnston, Lashkari, and Bernard. Johnston fails to disclose the use of a flavoring component with bacteria or enzymes present. Johnston discloses that one goal is to reduce variability in cheese making processes. ("Any method of cheese making that can reduce the variability and criticality of one of the traditional cheese making steps, yet maintain flexibility in the functional characteristics of the end cheese product, gives the cheese making industry a way of producing a cheese having the required functional characteristics in a consistent manner." Johnston at page 1, lines 18-22). There would be no reason to add the flavoring of Lashkari to Johnston because it has active bacteria and enzymes present that would introduce additional variables, uncertainty, and potential processing problems into the process of Johnston. The skilled artisan would also expect flavors to continue to develop in a ripened cheese having the flavoring component of Lashkari added to it. This could lead to undesired off flavors. For example, if the flavoring component of Lashkari was added to the mixture of Johnston there would be an expectation that the enzymes remaining in the sterilized fermented additive would continue to act on the lipids in the cheese and this would eventually produce off flavors in the product. *See* "Reports of gelation or development of bitter flavor during storage of sterile milk suggest the possible involvement of these enzymes." Speck and Adams at page 786; see also "Heat stable proteases produced by different psychotrophic Pseudomonas species attack all of the milk caseins and whey proteins leading to the development of bitter flavor and coagulation." *Id.* at 787. Johnston discloses that their

samples were held for 1 month at 5°C before organoleptic evaluation [Example 1] which is a typical maturation period for the enzymes present in traditional cheese to bring about ripening i.e. flavor production. Thus, a person of skill in the art would not combine the references as suggested because they would expect flavors to continue to develop, which would change the flavor, potentially unfavorably. Johnston is concerned with a controlled flavoring process and thus would not desire the continued flavor development that would occur in the proposed combination. Such a modification of Johnston would change the principle of operation of Johnston because rather than adding a known flavor, a continuous flavor development would occur.. If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). Applicants submit that there is no reason to make the combination of Johnston and Lashkari suggested in the Office Action and it would change the principle of operation of Johnston with respect to the flavor. Accordingly, Applicants respectfully request withdrawal of the rejection for at least this reason.

Further, this is also more than an obvious difference because the skilled artisan would not add the flavoring component of Lashkari to the process of Johnston because it would be expected to cause additional variability and possible processing problems. For example, the instant specification discloses that "A further known method is to standardize the flavor of such cheese by ripening the cheese to the optimal extent and then heat treating the cheese to arrest all microbiological processes. However, this process can cause undesirable flavor and unusual textural changes in the cheese. As a result, heat treated cheeses are often marked down in price" [paragraph 0008]; and also that: "Quality defects that take months to become evident represent expensive failures that the consumer ultimately carries" [paragraph 0011].

Applicants respectfully request withdrawal of the rejection of Claims 2 and 4 and their dependents because the combinations fails to disclose the features of Claims 2 and 4 and there is no reason to combine the cited references to achieve the recited features.

The combination of references fails to disclose or make obvious the features of Claim 3 and its dependents

Claim 3 recites mixing in a flavour concentrate containing viable organisms to form a cheese precursor and allowing the cheese precursor to ripen. Johnston fails to disclose the use of a flavor concentrate with viable organisms. Lashkari discloses sterilizing the flavor composition which kills only the viable organisms. The Examiner found Bernard to disclose a cheese product that is cooled with the surface of the cheese inoculated.

Applicants submit that there is no reason to combine Johnston, Lashkari, and Bernard as suggested in the Office Action. Johnston does not use a flavoring compounds with active bacteria or enzymes. Johnston discloses that one goal is to reduce variability in cheese making processes. ("Any method of cheese making that can reduce the variability and criticality of one of the traditional cheese making steps, yet maintain flexibility in the functional characteristics of the end cheese product, gives the cheese making industry a way of producing a cheese having the required functional characteristics in a consistent manner." Johnston at page 1, lines 18-22). There would be no reason to add bacteria or enzymes to Johnston or the flavoring composition of Lashkari to Johnston because it has active bacteria and enzymes present that would introduce additional variables, uncertainty, and processing problems into the process of Johnston. Further, mixing and matching the dairy based substrates, microorganisms, and techniques from Johnston, Lashkari, and Bernard is more than selecting from known techniques to achieve predicable results because of the uncertainty with chemical and biological processes. For example, as noted above, a person of skill in the art would expect off flavors to develop in a cheese produced by adding the sterilized flavor component of Lashkari, which would be expected to include active enzymes, to the substrate of Johnston and subsequently ripening the mixture. Thus, there is no reason to combine Johnston, Lashkari, and Barnard as suggested in the Office Action.

Applicants submit that there is no reason to make the combination of Johnston, Lashkari, and Bernard suggested in the Office Action. Applicants respectfully request withdrawal of the rejection of Claim 3 and its dependents.

The combination of references fails to disclose or make obvious the features of Claim 1 and its dependents

Claim 1 is amended herein to recite in part "forming a cheese that can be frozen and thawed while still maintaining a smooth texture." The Examiner found Johnston to disclose freezing the cheese at col. 10, line 26-27. Applicants note that Johnston discloses making mozzarella type cheeses and freezing the cheese in a block or as shredded cheese. The Examiner cited Chikuma to disclose freezing, thawing, and further ripening the curd at col. 3, lines 1-6). Chikuma discloses freezing curd in the context of separating the whey and dehydrating the curds ("the present invention relates to a process of cheese preparation comprising the step of freezing the curd and then melting the latter in water and pressing the same for the dehydration of curds." col. 1, lines 61-64).

Applicants also submit that there is no reason to combine for the reasons discussed above. Johnston is about controlling conditions with adding flavor compounds without organisms present. Adding flavoring with microorganisms would change the mode of operation of Johnston.

Freezing and thawing of cheese and the subsequent affect to the texture of cheese is not an issue that either Chikuma or Johnston is concerned with. Thus, there is no reason provided in Chikuma or Johnston to freeze and thaw a cheese. Adverse textural changes to cheese that is frozen are well known in the art. ("5. Freezing caused the texture of the cheese to be crumbly. The extent of crumbliness developed was dependent upon the texture and make-up of the cheese before freezing." Sommer, Abstract attached to this response and concurrently submitted in an IDS.) In addition, the instant specification discloses that "[i]t is also known that most cheese produced by conventional means cannot, in general, be stored frozen without major disruption to the texture of the curd mass." (paragraph [0010] of the specification as published). The specification further discloses that "[u]pon thawing, the flavour, aroma, and texture were surprisingly similar to a moderately ripened Blue cheese and showed no sign of serum separation or curd granularity." (paragraph [0116] of the specification as published). Chikuma and Johnston fail to provide any guidance as to how to successfully freeze and thaw cheese while maintaining a smooth texture. Applicants submit that there is no reason to combine Johnston, Lashkari, and Chikuma as suggested in the Office Action.

Thus, the presently claimed process results in a cheese that overcomes the adverse textural changes normally associated with freezing and thawing such as serum separation or curd granularity. Applicants submit that the combination of Johnston, Lashkari, and Chikuma fails to disclose or make obvious the features of Claim 1 and its dependents.

Applicants also submit that the cited references fail to disclose the concentrations recited in Claims 80-82 in combination with the other features of the claims. Applicants respectfully request withdrawal of the rejection of these claims for this reason as well.

The Combination of Johnston/Lashkari/Bernard/Chikuma Does Not Make Claims 7, 14, 24, 33, 39, 44, 47, 48, 54, 59, 61, 68, or 75 Obvious

The remaining claims stand rejected under Johnston and Lashkari in combination with one or more of Bernard, U.S. Patent 3,091,539 No. to Chikuma, U.S. Patent No. 2,965,492 to Bauman, The American Cheese Society publication, and U.S. Patent No. 4,655,127 to Skovhauge. Bauman, Chikuma, American Cheese Society publication, and Skovhauge fail to make up for the deficiencies noted above with respect to the combinations of Johnston and Lashkari and Johnston, Lashkari, and Bernard. Accordingly, Applicants submit that these claims are not made obvious for at least the reasons discussed above with respect to the combinations of Johnston/Lashkari, Johnston/Lashkari/ Bernard and Johnston/Lashkari/Chikuma.

Clams 5-9, 11-15, 18, 19, 22, 24, and 31-34, 36-49, 51-63, and 65-82 depend from Claims 1-4 and recite all the elements of Claims 1-4 in addition to reciting further distinguishing features. Thus, Applicants respectfully request withdrawal of the rejection of these claims as well, for at least the reasons set forth above.

Further, Applicants submit that the combinations also fail to disclose the features of Claims 7, 33, 47, and 61. Claims 7, 33, 47, and 61 depend indirectly from Claims 1, 2, 3, and 4, respectively and further recite wherein following the freezing step, the cheese is thawed and further ripening occurs. Applicants submit that the cited references fail to disclose or make obvious such features. Accordingly, Applicants respectfully request withdrawal of the rejections of Claims 7, 33, 47, and 61 for this reason as well.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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SYMPOSIUM: IMPACT OF HEAT STABLE MICROBIAL ENZYMES IN FOOD PROCESSING

Heat Resistant Proteolytic Enzymes from Bacterial Sources[1]

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Interest in the production of ultra-high temperature (UHT) sterilized milk has existed for a number of years. Recent economic and social developments could intensify interest in the UHT sterilization of a number of fluid dairy products. One of the main advantages of such products is that their freedom from viable microorganisms should permit their storage at room temperature and conserve energy costs by eliminating the need for refrigerated storage between processing and consumption. Changes in retailing practices, food service establishments, etc., also have presented new needs for sterilized products.

There is a variety of methods for sterilization of milk. It may be sterilized in bottles or cans at temperatures of 104 to 113 C for 12 to 35 min. However, such milk usually has a pronounced cooked flavor and a browned appearance (6). Sterilization at UHT is preferable because of reduced changes in appearance, flavor, and nutrients although problems of bitter flavor and gelation or coagulation can develop during storage (5, 11). UHT treatment directly by steam injection rather than indirectly by heat exchangers may minimize product damage further.

UHT treatments of 121 to 149 C for .5 to 8 s have been proposed for sterilizing milk (2, 12, 13). Speck and Busta (13) reported that 4 s at 149 C would yield at least a 12D reduction in spores of *Bacillus stearothermophilus* 1518 or Putrefactive Anaerobe (PA) 3679. These spores commonly are used for the development of sterilization processes, and a heat treatment of 149 C for 4 s should sterilize fluid milk products effectively.

There now is growing evidence that enzymes capable of spoiling milk can survive such UHT treatments (Table 1). Adams et al. (1) have reported that psychrotrophic bacteria isolated from raw milk produced proteases hundreds of times more heat resistant than the bacterial spores used by Speck and Busta (13). The time required to reduce protease activity by 90% was 90 s compared to .25 s and .02 s for a 90% reduction in PA3679 spores and *B. stearothermophilus* spores, respectively. These proteases suffered less than 10% destruction during UHT sterilization of milk at 149 C for 4 s. Inactivation data for a protease produced by *Pseudomonas fluorescens* P26 (9) indicated similar resistance as did the report by Bengtsson et al. (4). Production of heat-stable proteases was not restricted to select cultures or isolates. Such enzymes also were produced by the total psychrotrophic flora of raw milk during incubation at 4 C.

Production of heat-stable proteases by these psychrotrophs has been reported by at least four groups in the United States and Sweden in the past few years (1, 4, 8, 9). Psychrotrophs capable of producing heat-stable proteases have been isolated from raw milk in North Carolina (1) and Wisconsin (8). Adams et al. (1) found that 70 to 90% of the raw milk samples obtained from one dairy farm and two dairy plants contained psychrotrophs capable of producing heat-stable proteases. Production of such proteases by *Pseudomonas* species of unknown origin also has been reported in Missouri and Sweden. Reports of gelation or development of bitter flavor during storage of sterile milk suggest the possible involvement of these enzymes.

Although far from conclusive, data indicate that most raw milk supplies are likely to contain heat-stable proteases. Most, if not all, reports of heat-stable bacterial proteases identify gram negative psychrotrophs, usually *Pseudomonas* species, as the sources. Psychrotrophs probably are part of the flora of all normal raw milk, since it is difficult to exclude all psychrotrophs during production operations. Refrigeration, which is the primary method for controlling bacterial growth in raw milk, slows the growth of psychrotrophs when sufficiently low

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TABLE 1. Heat-stable bacterial enzymes.

Enzyme	Source	Heat resistance	References
Protease(s)	*Pseudomonas* (10 isolates)	$D_{150\ C}$ = 90 s	1
Protease(s)	Total psychrotrophic flora of raw milk	Survived 149 C/10 s	1
Protease	*P. fluorescens* P26	D_{149C} = 90 s[a]	9
Protease	*Pseudomonas*	Survived unspecified UHT sterilization treatment	4
Protease	*Pseufomonas*	Survived 135 C/3.5 min	8
Lipase(s)	*P. fluorescens* 22F	$D_{150\ C}$ = 4.8 min	7

[a]Calculated from data presented for inactivation at 63 C, 72 C, and 121 C.

temperature is used. However, it does not prevent their growth and metabolism over the long time which raw milk often is stored today. As milk processing operations become more centralized and raw milk spends more time in refrigerated storage before processing, the growth and metabolism of psychrotrophs can be expected to increase.

If proteases survive UHT sterilization, milk containing them and stored without refrigeration would be a good environment for their activity. The optimum pH for one protease ranged from pH 7 to 8 with 85 to 90% of maximum activity at pH 6.5, the pH of milk (1). Optimum casein concentration was 2%, but activity was about 80% as high at 2.5% casein and about 75% at 3.5% protein as casein (1). The only other environmental variable for milk would be the storage temperature. Adams et al. (1) reported that the protease produced by psychrotrophs of dairy origin was most active at 45 C, but activity was 25% of maximal at normal room temperature. Milk stored without refrigeration could be exposed to much higher temperatures which would be nearer the optimum for the enzyme activity of 40 to 45 C. The long storage expected of sterile milk allows for even low concentrations of surviving enzymes to act. Also, storage increases the susceptibility of the milk proteins to protease attack. The extent of proteolysis that occurred during 1 h after the addition of protease to milk stored at room temperature (25 C) for 55 days was more than twice that which occurred with freshly UHT treated milk. After an additional 36 days of storage, the milk coagulated upon the addition of protease (1).

Heat stable proteases produced by different psychrotrophic *Pseudomonas* species attack all of the milk caseins and whey proteins leading to the development of bitter flavor (1, 9) and coagulation (1). We have found that UHT sterilized milk inoculated with .19 units of protease developed a bitter flavor within 30 days during storage at 40 C (1). Mayerhofer et al. (9) reported that sterile milk inoculated with .2 unit of *Pseudomonas fluorescens* P26 protease (equivalent of 266 of our protease units) developed bitter flavor within 30 days at 4 C. Detection of low concentrations of protease in milk is difficult and will require development of much more sensitive assay procedures, but there is some evidence that psychrotrophic populations under 10,000 per ml can produce about 10 or more units of heat stable protease per milliliter which would shorten the shelf-life of sterile milk significantly.

These proteases also may influence greatly the shelf-life of other food products. White and Marshall (14) found that protease had no effect on butter quality, but flavor scores of cottage cheese and cheddar cheese containing protease were significantly lower than control cheeses. For ice cream mix, statistically significant evidence of proteolysis was obtained with samples containing protease. This was not reflected in taste panel studies, possibly because of the inherent pronounced natural flavor of ice cream. Survival of heat stable proteases in dry milk, sterile milk, or other dairy products could affect deleteriously certain formulated foods containing milk. Swanson (personal communication) reported the development of bitter flavor in baby food containing sterile milk.

TABLE 2. Methods for controlling heat-stable proteases in milk.

Method	Problem
1) Prevent contamination by psychrotrophs	Difficult, expensive
2) Prevent growth and metabolism by psychrotrophs	
a) Lower temperature and decrease aeration	Possibly difficult and expensive
b) Add antibiotics or other inhibitors	Not allowed
c) Add starter organisms that produce inhibitors	Possible off-flavors
3) UHT inactivation	Undesirable changes in flavor and color, loss of nutrients
4) Inactivation at sub-pasteurization temperatures	None known

The foregoing indicates that the successful use of UHT treatment of milk can be hindered by heat stable enzymes in milk. The industry appeats to be in a dilemma in having to cope with enzymes more heat stable than the most heat resistant bacterial spores. It is necessary, therefore, to find means to prevent their addition to milk or to discover methods to inactivate them.

Controlling the protease in raw milk presents problems (Table 2). Assuring low psychrotroph populations in milk to be UHT sterilized would be difficult to accomplish and would be expensive. The addition of antibiotics or other inhibitors is unacceptable. Starter culture bacteria have inhibited *Pseudomonas* species (10). Such organisms might be added to raw milk supplies to control the growth of psychrotrophs so long as they themselves do not affect the milk. Lowering the temperature or decreasing the aeration of the milk also are possible alternatives.

Ideally, measures to control heat stable proteases should be applied in the processing plant where close and positive control could be maintained. However, destruction of these proteases at ultra-high temperatures would require severe treatments that would be deleterious to the milk. Extrapolation of data for UHT treatments to lower temperatures indicated (1) that at these temperatures very long treatment times would be required, e.g., 6 h at 72 C, which could be equally damaging and certainly would be uneconomical. Barach et al. (3) reported recently, however, that heat-stable proteases in milk could be inactivated at sub-pasteurization temperatures, apparently by some mechanism other than heat. At 55 to 60 C the amount of surviving protease was 8 to 22 times less than expected. Heat-stable proteases of eight other psychrotrophs isolated from raw milk behaved similarly. This low temperature inactivation occurred equally well with protease in raw or sterile milk with about 70% destruction in 1 h. Low temperature inactivation appeared to be independent of the concentration of the protease in the milk. This would be important, since protease concentration in milk probably would be variable. The effectiveness of the low temperature inactivation over a wide range of protease concentrations also indicated that it would be effective at the low protease concentrations expected in normal milk.

To be of value, the low temperature treatment would have to destroy the proteases and not deleteriously affect the milk itself. Barach et al. (3) reported that the low temperature destruction of high concentrations of heat-stable protease in raw or sterile milk did not alter the flavor or protein content of the milk. The κ-casein was most susceptible to the protease tested but was not affected by the low temperature treatment. Such a process, if feasible on a commercial scale, could offer the best solution to the problem presented by heat-stable proteases.

At the present time, UHT treatment of milk can be successful only if the presence or activity of heat stable proteases can be controlled. Studies currently are being undertaken to develop procedures that will control such enzymes in milk, as well as the method for their inactivation.

ACKNOWLEDGMENT

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The Freezing Point of Cheddar Cheese: Injury of Cheese by Freezing*

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Summary

1. The freezing point of cheese can be determined with sufficient accuracy by the use of a 1-inch cube of cheese and a suitable thermometer.
2. The freezing points found for 15 different Cheddar cheeses ranged from -4.3° to -14.3°C.
3. In addition to the moisture and salt content, the age of the cheese is an important factor in the freezing point.
4. The cheese subjected to freezing showed no perceptible injury to the flavor.
5. Freezing caused the texture of the cheese to be crumbly. The extent of crumbliness developed was dependent upon the texture and make-up of the cheese before freezing.
6. Freezing caused the paraffin to flake off from paraffined cheese.
7. On storage at favorable temperatures after freezing, the cheese texture recovered, in some cases apparently completely.
8. After the texture had recovered, distinction between frozen and unfrozen cheese could be made on the basis of the manner in which the cheese checked on drying at the surface.

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